BMP-12 and BMP-13 transduced MSCs in collagen hydrogel for ACL reconstruction
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Introduction: With increased participation in sports, the frequency of anterior cruciate ligament (ACL) ruptures is rapidly increasing. Currently over 200,000 patients rupture their ACL each year in the USA [1]. The ACL fails to heal after rupture, and loss of ACL function leads to knee instability, loss of proprioceptive function, and osteoarthritis in over 60% of patients. Ligament reconstruction with biologic grafts such as autologous patellar tendon or hamstring tendon is the gold standard but does not restore the complex architecture and biomechanics of the ACL. A number of growth factors such as transforming growth factor (TGF-β), insulin-like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), and respectively, bone morphogenetic proteins-12 and -13 (BMP-12 and -13) have been evaluated for their ability to stimulate different aspects of ligament repair, including outgrowth, cell division, and collagen synthesis [2,3]. In this study the ligament differentiation of human mesenchymal stem cells (hMSCs) transduced with BMP-12 and embedded in collagen hydrogel was investigated.

Materials and Methods: Human MSCs from bone marrow were transduced at confluency with 10 and 100 vp/cell of adenovirus encoding BMP-12 and BMP-13, respectively. Afterwards, genetically modified hMSCs were placed at 3 x 10^5 cells per 200 μl collagen type I hydrogel (Arthro Kinetics plc, Esslingen, Germany). After 21 days in culture histochemical (Azan, van Gieson, and Masson Goldner staining), immunohistochemical (collagen type III and V, elastin, fibronectin, tenascin, and vimentin), and RT-PCR analyses of the hydrogel constructs were performed.

Results: Histochemical (H&E and Masson Goldner) revealed elongated fibroblast-like cells in the collagen hydrogel (Fig. 1). MSCs transduced with 10 vp/cell BMP-12 and -13, respectively showed a higher cell number than with 100 vp/cell. A ligament-like matrix was observed with immunohistochemical analyses (Fig. 2). The genetical modification of MSCs with BMP-12 resulted in a moderate staining for collagen type III and V and a weak for elastin. In contrast, BMP-13 transduced MSCs showed a weak staining for collagen type III and V and elastin.

A strong staining for tenascin and vimentin was detected for BMP-12 and BMP-13 transduced MSCs, respectively (Fig. 2). Furthermore, specific ligament markers like biglycan, collagen type III and V, decorin, elastin, tenasin, tenomodulin, and vimentin were expressed as shown in the RT-PCR analyses.

Discussion: Gene transfer with BMP-12 and BMP-13 in combination with a collagen type I hydrogel lead to the development of a ligament-specific extracellular matrix. Therefore, the use of genetically modified hMSCs could be an effective strategy for the formation of genetically optimized hydrogel constructs for the ligament repair.


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